Anatomical phenotyping of the PML knockout mouse

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Introduction: The promyelycitic leukemia protein (PML) is a growth suppressor that regulates the tumour suppressors p53 and pRb. Previously it has been shown that Pml expression is lost in various human tumours, including tumours of the central nervous system. More recent work with a PML knock out mouse has also revealed a role of PML in neurogenesis. In this respect, PML expression is restricted to neural progenitor cells (NPCs) in the developing neocortex. A loss of PML causes an increase in the number of NPCs, and skews the composition of NPC subtypes in the neocortex, ultimately resulting in impaired neuronal differentiation. The consequences of this abnormal development have been histologically assessed at postnatal day 0 (P0), by which time neurogenesis is complete[1]. It was found that the cortex was considerably reduced in size, and anatomical landmark measurements in the hippocampus showed this structure also to be affected. What remains to be established is whether developmental changes induced by PML loss results in alterations of brain size in the adult animal. To address this point we apply tensor-based morphometry(TBM) to magnetic resonance images of P28 control and $PML^{-/-}$ mice. This allows us to determine whether the same regions are affected at this later stage of development, and further to screen the entirety of the brain for additional regions which may be affected by the loss of PML.

Methodology:
Image acquisition: 7 $PML^{-/-}$ and 4 $PML^{+/-}$mice, aged 28 days, were perfuse-fixed and decapitated. Excess tissue and bone structures were removed and the intact skulls were post-fixed in a solution of 4% formal-saline and 8mM Gd-DTPA for 9 weeks. Skulls were then imaged on a Varian 9.4T VNMRS system with a 26mm quadrature volume coil using a 3D spoiled gradient echo sequence. Parameters: TE=4.03ms, TR=17ms, FA=52^o, FOV=20.48x13.04x13.04mm^3, Matrix=512x326x326, Averages=6, Scan time=3 hours[2].
Image processing: The 11 brains were group-wise registered, and their average taken to produce a high SNR atlas. The group-wise registration consisted of two iterations of a block-matching affine registration algorithm[3], followed by 10 iterations of a free-form deformation(FFD) algorithm[4]. The FFD registration was constrained as to heavily penalise any biologically inconsistent folding, resulting in registered images with positive Jacobian determinants at all voxels. At each voxel of the atlas, and for each subject, the determinant of the Jacobian (a direct measure of volume change between atlas and source image) was calculated using the transformation models determined by the registration. Statistical parametric mapping software (SPM8, http//www.fil.ion.ucl.ac.uk/spm) was used to statistically compare the determinant of the Jacobian between groups at each voxel of the atlas, thus producing a map of regional volume differences between Tc1 and control groups.

Results: Widespread regions of reduction were observed, particularly in the cortex and hippocampus. Figure 1 localises the cortical reductions to the occipital lobes and the posterior part of the parietal-temporal lobes. Figure 2 demonstrates that large regions of the hippocampus are also reduced in volume.

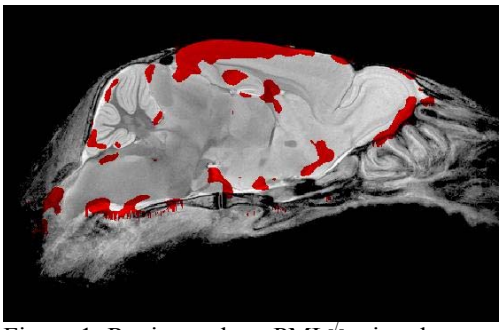

Figure 1: Regions where $PML^{-/-}$ mice show a significant ($p<0.05$, corrected by false discovery rate of 0.05) decrease in volume are red.

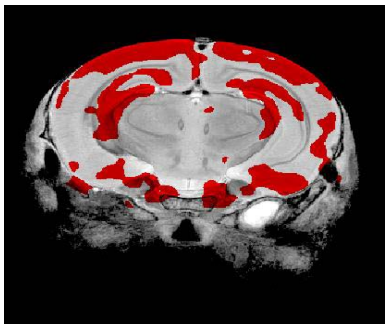

Figure 2: Regions where $PML^{-/-}$ mice show a significant decrease in volume are red.

Discussion: Although these results are drawn from a small, pilot sample of 11 subjects, we already attain statistical significance in the particularly affected regions. The TBM analysis demonstrates that the cortical and hippocampal deficits reported for P0 mice are still present at P28. This reduction is attributed to an impaired transition between radial glial cells and basal progenitors, which in turn results in a reduced differentiation and an overall decrease in the thickness of the cortex wall. We did not however find a statistically significant reduction in total brain size, which is seen for the P0 mice. This may be due to postnatal compensation in brain development between days 0 to 28, but is more likely due to an insufficient sample size to reach significance. In the near future we will increase our sample to 11 $PML^{-/-}$ and 11 $PML^{+/-}$. We will also expand on the analysis by making automated volume measurements of the hippocampus and the cortical lobes, and measure cortical thickness at each point on the surface of the cortex.

References: [1] Regad et al. Nature Neuroscience (2009), vol 12, p132-140, [2] Cleary JO et al. 2010 Proc ISMRM #1044, [3] Ourselin et al. Image and Vision Computing (2000), vol 19 , p25-31, [4] Modat et al. Comput Meth Prog Bio (2010), vol. 98 (3), p278-84